AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the application.

1-8. (Canceled).

9. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of

a) thermally cycling a PCR ~~mixture~~ mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m;

b) obtaining during each thermal cycle ~~by cycle~~ a first emission reading of the double stranded DNA intercalating dye at a first measuring ~~temperature~~ temperature, wherein the first measuring temperature is between an annealing/extension temperature and the first T_m, ~~first T_m~~ and a second emission reading of the double stranded DNA intercalating dye at a second measuring ~~temperature~~ temperature, wherein the second measuring temperature is between the first T_m and the second T_m;

c) determining during each thermal cycle ~~by cycle~~ a first emission amount of the first amplicon which is the difference between the first emission reading and the second emission reading, and a second emission amount of the second amplicon which is the second emission reading.

10. (Canceled).

11. (Canceled).

12. (Currently Amended) The method of claim 9, ~~claim 11~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

13. (Canceled).

14. (Canceled).

15. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the first measuring temperature is 0.25°C below the first T_m, 0.5°C below the first T_m, 1.0°C below the first T_m, 1.5°C below the first T_m, or 2.0°C below the first T_m, and wherein the first measuring temperature is higher than the annealing temperature.

16. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is 0.25°C below the second T_m, 0.5°C below the second T_m, 1.0°C below the second T_m, 1.5°C below the second T_m, or 2.0°C below the second T_m, and wherein the second measuring temperature is higher than the first T_m.

17. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is 0.25°C above the first Tm, 0.5°C above the first Tm, 1.0°C above the first Tm, 1.5°C above the first Tm, or 2.0°C above the first Tm, and wherein the second measuring temperature is less than the second Tm.

18. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the second measuring temperature is the first T_m + 0.25°C< the second measuring temperature < the second T_m-0.25°C, the first T_m + 0.5°C< the second measuring temperature < the second T_m-0.5°C, the first T_m + 1.0°C< the second measuring temperature < the second T_m-1.0°C, the first T_m + 1.5°C< the second measuring temperature < the second T_m-1.5°C, or the first T_m + 2.0°C< the second measuring temperature < the second T_m-2.0°C.

19. (Canceled).

20. (Currently Amended) The method of claim 9, ~~claim 9~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading.

21. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of:

a) thermally cycling a PCR ~~mixture~~ mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m;

b) obtaining during each thermal cycle ~~by cycle~~ a first pre-T_m emission reading of the double stranded DNA intercalating dye at a measuring temperature which is below the first T_m and a first post-T_m emission reading of the double stranded DNA intercalating dye at a ~~at the~~ measuring temperature which is above the first T_m and a second pre-T_m emission reading of the double stranded DNA intercalating dye at a measuring temperature which is below the second T_m and a second post-T_m emission reading of the double stranded DNA intercalating dye at the a measuring temperature which is above the second T_m;

c) determining during each thermal cycle ~~by cycle~~ a first emission amount of the first amplicon which is the difference between the first pre-T_m emission reading and the first post-T_m emission reading; and a second emission amount of the second amplicon which is the difference between the second pre-Tm emission reading and the second post-T_m emission reading.

22. (Canceled).

23. (Currently Amended) The method of claim 21, ~~claim 22~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

24. (Canceled).

25. (Canceled).

26. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature below the first T_m and/or the second T_m are 0.25°C below, 0.5°C below, 1.0°C below, 1 .5°C below, or 2.0°C below.

27. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature above the first T_m and/or the second T_m are 0.25°C above, 0.5°C above, 1.0°C above, 1.5°C above, or 2.0°C above.

28. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing the calculation of subtracting the first pre-T_m emission reading from the first post-T_m emission reading or subtracting the first post-T_m emission reading from the first pre-T_m emission reading, and the second emission amount of the second amplicon is obtained through the computer program performing the calculation of subtracting the second pre-T_m emission reading from the second post-T_m emission reading or subtracting the second post-T_m emission reading from the second pre-T_m emission reading.

29-84. (Canceled).

85. (Currently Amended) A method for real-time detecting and quantifying a first ~~nucleic acid template~~ amplicon and a second amplicon ~~nucleic acid template~~ in a PCR mixture comprising the steps of

a) thermally cycling a PCR mixture, ~~mixture~~ wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, the first template and the second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m;

b) obtaining during each thermal cycle ~~by cycle~~ a first emission reading of the double stranded DNA intercalating dye at a first measuring temperature, wherein the first measuring temperature is between an annealing/extension

temperature and the first T_m, a second emission reading of the double stranded DNA intercalating dye at a second measuring temperature, wherein the second measuring temperature is between the first T_m and the second T_m; and a third emission reading of the double stranded DNA intercalating dye at a third measuring temperature, wherein the third measuring temperature is between the second T_m and a total denaturing temperature; and

c) determining during each thermal cycle ~~by cycle~~ a first emission amount of the first amplicon which is the difference between the first emission reading and the second emission reading, and a second emission amount of the second amplicon which the difference between the second emission reading and the third emission reading.

86. (Canceled).

87. (Currently Amended) The method of claim 85, ~~claim 86~~ wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

88. (Canceled).

89. (Canceled).

90. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the first measuring temperature is 0.25°C below the first T_m, 0.5°C below the first T_m, 1.0°C below the first T_m, 1.5°C below the first T_m, or 2.0°C below the first T_m, and wherein the first measuring temperature is higher than the annealing temperature.

91. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is 0.25°C below the second T_m, 0.5°C below the second T_m, 1.0°C below the second T_m, 1.5°C below the second T_m, or 2.0°C below the second T_m, and wherein the second measuring temperature is higher than the first T_m.

92. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is 0.25°C above the first T_m, 0.5°C above the first T_m, 1.0°C above the

first T_m, 1.5°C above the first T_m, or 2.0°C above the first T_m, and wherein the second measuring temperature is less than the second T_m.

93. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the second measuring temperature is the first T_m + 0.25°C < the second measuring temperature < the second T_m-0.25°C, the first T_m + 0.5°C < the second measuring temperature < the second T - 0.5°C, the first T_m + 1.0°C < the second measuring temperature < the second T_m-1.0°C, the first T_m + 1.5°C < the second measuring temperature < the second T_m-1.5°C, or the first T_m + 2.0°C < the second measuring temperature < the second T_m-2.0°C.

94. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the third measuring temperature is 0.25°C above the second T_m, 0.5°C the second T_m, 1.0°C above the second T_m, 1.5°C above the second T_m, or 2.0°C above the second T_m, and wherein the third measuring temperature is less than the total denaturing temperature.

95. (Currently Amended) The method of claim 85, ~~claim 85~~ wherein the first emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading, and the second emission amount of the second amplicon is obtained through a computer program performing a calculation of subtracting the second emission reading from the third emission reading or subtracting the third emission reading from the second emission reading.

96. (Currently Amended) The method of claim 21, ~~claim 21~~ wherein the measuring temperature above the first T_m and the measuring temperature below the second T_m ~~is the~~ are the same.